Synthesis of Chondroitin Sulfate D and Heparin Proteoglycans in Murine Lymph Node-derived Mast Cells

THE DEPENDENCE ON FIBROBLASTS*

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Proteoglycans synthesized in cultured mast cells derived from horse serum-immunized lymph node cells were analyzed. Treatment of the 35S-proteoglycans extracted from these cells with either chondroitinase ABC or AC resulted in 95% ± 7% and 84% ± 7%, respectively (mean ± S.E., n = 3), of the radioactivity associated with disaccharides eluting in the included volume of PD-10. The 35S-proteoglycans were not hydrolyzed by nitrous acid elimination treatment. The chondroitinase ABC-generated disaccharides were analyzed by aminocyano high performance liquid chromatography. 35S-Disaccharides eluted in a major peak at a retention time of 8.1 min, corresponding to the disaccharide of chondroitin 4-sulfate proteoglycan (ΔDi-4S), and a second peak at 12 min, corresponding to the disaccharide of chondroitin sulfate D proteoglycan (ΔDi-diS). Further treatment with chondro-4-sulfatase Δ not affect the retention time of the disaccharide corresponding to ΔDi-diS, whereas this peak disappeared after the digested proteoglycan was treated either by chondro-6-sulfatase or by both sulfatases. Therefore, this disaccharide was identified as chondroitin sulfate D. Quantification of the radiolabeled disaccharides showed that ΔDi-diS contributed 20% ± 2% (n = 3) of the total sulfated disaccharides of the chondroitin sulfate of these cultured cells. The role of fibroblasts in inducing the shift of chondroitin sulfate D into heparin proteoglycan in these mast cells was also investigated by using three types of monolayers: mouse embryonic skin fibroblasts (MESF), rat embryonic skin fibroblasts (RESF), and 3T3 fibroblasts. 35S-Proteoglycans that were extracted from the lymph node-derived mast cells cultured for 30 days on MESF and on 3T3 fibroblast monolayers were 93% ± 4% and 30% ± 7% (n = 3) susceptible to nitrous acid elimination, respectively. No degradation by nitrous acid was observed in 35S-proteoglycans extracted from cells cultured on RESF monolayer. Since the MESF was found to be the most potent monolayer in the induction of heparin synthesis, the kinetics of changes in the synthesis of proteoglycan types were determined in lymph node-derived mast cells cultured on MESF for up to 30 days. It was found that the synthesis of chondroitin sulfate gradually declined whereas that of heparin starting between 4 and 7 days after plating gradually increased. From the 17th day on, only the synthesis of heparin was detected.

Oversulfated proteoglycans have become useful phenotypic markers for distinguishing subclasses of mast cells. Heparin proteoglycan was first described in the secretory granules of connective tissue mast cells derived from rat (1) and mouse (2) peritoneal cavity. Later it was also identified in human lung (3) and skin mast cells (4). Chondroitin sulfate E (ChS-E),1 an oversulfated proteoglycan of about M, 250,000, possessing disulfated disaccharides GlcUA → GalNAc-4, 6-diSO4 (ΔDi-diS) was identified in the secretory granules of cultured mouse bone marrow-derived or mouse fetal liver-derived, interleukin 3-dependent mast cells (2, 5, 6). These cultured mast cells are the analogues of ChS-E containing mucosal mast cells identified in vivo in the mucose membranes of human colon (7) and stomach (8) and later in human lung (9). ChS-E was also found in the secretory granules of cultured human bone marrow-derived mast cells (10). Another oversulfated chondroitin sulfate proteoglycan chondroitin sulfate B, (ChS-diB), was identified in the secretory granules of rat bone marrow-derived mast cells (11). The different mast cell subclasses differ in a wide range of other properties. A most important aspect of this heterogeneity is the dependence of cultured mast cells on factors derived from T cells for differentiation and growth (5, 6, 12).

The interrelationship between these two cell subclasses of mast cells has been extensively investigated. We have shown that signals derived from the mouse embryonic skin fibroblasts (MESF) induce a phenotype change in cultured mast cells derived from immune lymph nodes (LNMC) into connective tissue-like mast cells (13, 14). These phenotypic changes were confirmed later in other culture systems in which ChS-E containing mouse bone marrow-derived mast cells cultured on mouse skin-derived 3T3 fibroblast cell line synthesized a significant amount of granular heparin proteoglycan (15). These studies have been extended by Kitamura and co-workers (16, 17), who provided evidence in vivo which

1 The abbreviations used are: Chs-E, chondroitin sulfate E; Chs-D, chondroitin sulfate D; Chs-diB, chondroitin sulfate B; HPLC, high performance liquid chromatography; LNMC, lymph node-derived mast cells; MESF, mouse embryonic skin fibroblasts; RESF, rat embryonic skin fibroblasts; ΔDi-4S, disaccharide of chondroitin 4-sulfate proteoglycan; ΔDi-6S, disaccharide of chondroitin 6-sulfate proteoglycan; ΔDi-diS, disaccharide of chondroitin sulfate E proteoglycan; ΔDi-diS, disaccharide of chondroitin sulfate D proteoglycan; ΔDi-diS, disaccharide of chondroitin sulfate B proteoglycan; GlcUA, glucuronic acid.
indicates that the mast cell can acquire phenotypic characteristics depending upon the environmental signals. Therefore, T cell growth-dependent mast cells may function as precursors of both types of cells. Most recently, mice mesenteric lymph node-derived mast cell committed precursors have been reported to acquire a serosal (connective tissue) mast cell phenotype when cultured on monolayers of either 3T3 fibroblast cell line or MESF or when cultured on methylocellulose in the presence of medium conditioned by these monolayers (18). On the other hand, these mouse lymph node-derived mast cell committed precursors acquire a mucosal mast cell phenotype when cultured in the presence of mouse interleukin 3.

In a previous study on mouse LNMC cultured in the presence of medium conditioned by the WEHI-3B myelomonocytic cell line, which releases mouse interleukin 3, we have analyzed the granular proteoglycan of these cells (19). We found that chondroitinase ABC-digested proteoglycan yielded two disaccharides that co-migrated on ascending thin layer chromatography with ΔDi-4S and ΔDi-diSz standards. By using this criterion, without any further analysis, it was assumed that the LNMC granular proteoglycan is Chs-E. The cells were therefore considered cultured analogues of mucosal mast cells.

In the present study we have reanalyzed the disaccharide composition of the glycosaminoglycans of this LMC-derived proteoglycan using additional methods. It was found that these glycosaminoglycans contain disulfated disaccharides GlcUA-2-SO₄, GalNAc-6-SO₄, termed chondroitin sulfate D (Chs-D) rather than Chs-E as described previously (19). In order to investigate whether these Chs-D-containing mast cells change their phenotype depending upon their microenvironment (in the same way that mouse bone marrow-derived mast cells depend on 3T3 fibroblasts), further work has been done. The role of three different fibroblast monolayers in triggering synthesis of heparin proteoglycan in these mast cells was investigated. Out of the three types of monolayers (MESF, RESF, and 3T3 fibroblasts), the MESF monolayer was found to be far superior in causing such an induction.

**MATERIALS AND METHODS**

**Preparation of LMC—**The procedure for producing a 98% homogeneous population of LNMC has been described previously (13). Briefly, BALB/c female mice 1–2 months old were injected intraperitoneally with 0.2 ml of horse serum once a week for 5 weeks. Two to seven days later 15 × 10⁶ mesenteric lymph node cells from immunized mice were cultured in the presence of Dulbecco's modified Eagle's medium supplemented with 10% (v/v) horse serum and 10% conditioned medium (cultured medium) in tissue culture tubes (Falcon, Becton Dickinson). The conditioned medium was prepared by culturing mesenteric lymph node cells derived from cercaria-infected mice (13). After 14 days of incubation, 98% of the homogenous population of LNMC was identified. Then, 3 × 10⁶ LNMC were cultured for various periods of time in 3 ml of cultured medium on different types of fibroblast monolayers prepared on 35-mm Petri dishes (Falcon).

**Preparation of Fibroblast Monolayers—**MESF was prepared from skin of 19-20-day-old BALB/c mouse embryos as described previously (20). RESF was prepared from Sprague-Dawley rat embryos (19-20 days) by the same procedures described for the preparation of MESF (21, 22). The Swiss-albino mouse skin-derived 3T3 fibroblast cell line was obtained from the American Type Culture Collection, Rockville, MD. The fibroblasts were x-irradiated with a cytotoxic dose of 5000 rad and then cultured in Waymouth medium (20) in concentrations ranging from 0.06 × 10⁶ to 1 × 10⁶ cells/plate. The fibroblasts remained viable yet did not divide. The cells continued to synthesize proteoglycans, which were analyzed in monolayers prior to co-culture and in fibroblasts separated from the co-culture after 30 days.

**Separation of LNMC from Fibroblasts—**The cells from each plate were dispersed by 0.1% trypsin, washed twice, and resuspended with 30 ml of Dulbecco's modified Eagle's medium containing 15% horse serum. The cell suspension was then plated into three tissue culture dishes (100 × 20 mm) (Falcon). After a 1-h incubation at 37 °C, CO₂ incubator, most of the fibroblasts adhered to the plates whereas the mast cells remained in suspension and were enriched up to a purity of 94% ± 6% (mean ± S.E., n = 6) as determined by toluidine blue staining.

**Preparation of Radiolabeled Proteoglycan**—Proteoglycans were characterized in each cell type separately both prior to the co-culture and after separation from the co-culture. The LNMC-associated proteoglycan was characterized prior to co-culture in cultures that contained more than 94% mast cells and in mast cells separated and enriched from the co-cultures (as described above) after the different co-culture conditions. The fibroblast-associated proteoglycan was assayed from all three monolayers prior to the co-culture, as a base line, and after the fibroblasts were separated from the co-culture at the times indicated.

The procedure of proteoglycan analysis was as follows. Cells were incubated for 4 h at 37 °C in fresh culture medium containing 100 μCi/ml [35S]sulfate (4000 Ci/mmol) (Du Pont-New England Nuclear). The radiolabeled cells were sedimented at 400 × g, resuspended in 0.2 ml of 0.05 M sodium acetate containing 1% (w/v) picric acid, and incubated for 4 h at 37 °C. The radiolabeled proteoglycans were purified by separation over a 45 × 0.8-cm Sepharose 4B column (Pharmacia) equilibrated and eluted in TSG buffer. Half-milliliter fractions were collected, and the radioactivity was determined in a B-spectrometry counter. 35S-labeled proteoglycans were purified by separation over a 45 × 0.8-cm Sepharose 4B column (Pharmacia) equilibrated and eluted in phosphate-buffered saline, pH 7.4. One-milliliter fractions were collected and radioactivity peak separated, dialyzed overnight against water, and lyophilized. Samples were resuspended in phosphate-buffered saline containing 0.2% trypsin, (0.01%), and incubated for 1 h at 37 °C. The proteoglycans were then purified further by CsCl density gradient ultracentrifugation under dissociative conditions by adding CsCl to a starting density of 1.4 g/ml in TSG buffer to the cell extracts and centrifuged at 95,000 × g for 48 h at 20 °C. CsCl gradients were divided into three fractions. The bottom fraction, which contained 80% of the 35S-labeled macromolecules, was dialyzed for 1 day against water and lyophilized. The purified proteoglycans were resuspended in 500 ml of TSG buffer and applied to PD-10 columns. Twenty 0.5-ml fractions were collected, and the radioactivity was quantified. 35S-Proteoglycans eluted at the PD-10 void volume (V₀) were collected, dialyzed for 1 day against water, and lyophilized. The presence of 35S-labeled heparin and 35S-labeled chondroitin sulfate glycosaminoglycans was assessed by determining the susceptibility of the proteoglycans to degradation by nitrous acid elimination and chondroitinase ABC and AC digestion. Nitrous acid susceptibility was determined as described previously (2). Briefly, 35S-labeled glycosaminoglycans were incubated for 24 h at −20 °C in the presence of 1,2-dimethoxyethane and butyl nitrite (Eastman Kodak). Authentic [1H]heparin (Du Pont-New England Nuclear) was used as control. Elimination products were chromatographed on the Sephadex G-25/PD-10 columns, with degradation being assessed by determining the shift in 35S radioactivity from the V₀ to the included volume. For the determination of glycosaminoglycan susceptibility to degradation by chondroitinase ABC or AC, peaks of the purified radiolabeled proteoglycans were resuspended in 1 ml of Tris-HCl buffer, pH 8.0, composed of 50 mM Tris-HCl, 50 mM NaCl, 35 mM sodium acetate, and 0.5% bovine serum albumin with or without 0.4 unit of either chondroitinase ABC or AC (Sigma) for 4 h at 37 °C (2). Cold chondroitin 4-sulfate and chondroitin 6-sulfate (5 μg each) (Sigma) were used as carriers to reduce contamination of radioactivity. Radioactivity in the bone marrow-derived mast cell-derived 35S-labeled chondroitin sulfate E proteoglycan was used as control. Digests were chromatographed on the Sephadex G-25/PD-10 columns, with degradation being assessed by determining the shift in 35S radioactivity from the V₀ to the included volume.

The macromolecules of the chondroitin sulfate disaccharides were analyzed by aminoacyl HPLC by a method described previously (23) and by their mobilities on ascending thin layer chromatography on precoated cellulose acetate plates (19). Chondroitin-6-sulfate (0.002 unit/μg of carrier), chondroitin-4-sulfate (0.002 unit/μg of carrier), or...
both sulfatases sequentially in a specific order were added to some samples after the chondroitinase ABC digestion (2). The same treatment was performed with [35S]ChS-E proteoglycan (2) derived from cultured mouse bone marrow-derived mast cells and for ChS-D proteoglycan derived from shark cartilage (kindly provided by Dr. Seno, Ochanomizu University, Tokyo, Japan). For HPLC analysis, [35S]-labeled proteoglycans were digested by chondroitinase ABC and AC or chemically degraded in 80% ethanol, cooled to 4°C for 2 h, and then centrifuged in Beckman Microfuge at 8000 x g for 5 min. The supernatant was decanted, dried over nitrogen, and resuspended in the HPLC solvent, which was 70% acetonitrile/methanol (3:1, v/v) and 30% 0.5 M ammonium acetate/acetatic acid, pH 5.3, with an apparent final pH of 7.0. A 4.6 x 250-mm Partisil-10 PAC aminoacyano column with a 4.6 x 25-mm precolumn containing the same packing (Whatman) was used for separating disaccharides. Eluates were collected for 0.5-min intervals and quantified by β-scintillation counting. The following disaccharides were used for standards: ΔDi-6S and ΔDi-4S (Miles Laboratories), [35S]ΔDi-diS₅ (mouse bone marrow-derived mast cells) (2), [35S]ΔDi-diS₀ (rat basophilic leukemia cells) (11), and ΔDi-diS₀ (shark cartilage). These standards, which were run each time, were detected either by continuous monitoring of ultraviolet absorbance at 232 nm or by determining the 35S radioactivity. The retention times of the disaccharide standards were: ΔDi-6S, 5.8 min; ΔDi-4S, 8.1 min; ΔDi-diS₀, 12 min; ΔDi-diS₅, 14.2 min; ΔDi-diS₀, 15.9 min; and free 35S, 24–26 min. The disaccharides that were liberated by chondroitinase ABC digestion were and were treated further by the sulfatases were characterized by their mobility relative to disulfated and monosulfated disaccharide standards on HPLC.

**RESULTS**

**Disaccharide Composition of the Glycosaminoglycan Side Chains of LNMC Proteoglycans**—Purified [35S]-labeled proteoglycans extracted from LNMC were either enzymatically digested with chondroitinase ABC or AC or chemically degraded by nitrous acid elimination. The percentages of disaccharides liberated by each procedure were quantitated by Sephadex G-25/PD-10 chromatography. In the untreated control sample, all radioactivity of 35S-proteoglycans was filtered in the void volume of the PD-10 column (Fig. 1a). Treatment with either chondroitinase ABC or AC resulted in 95% ± 7% and 84% ± 7%, respectively (n = 3), of the total radioactivity being associated with disaccharides eluting in the included column volume (Fig. 1, b and c). The [35S]-proteoglycans were not degraded by nitrous acid elimination treatment (Fig. 1d). The chondroitinase ABC-generated unsaturated disaccharides derived from the LNMC proteoglycans were analyzed first by thin layer chromatography. These disaccharides migrated in the position of ΔDi-4S and ΔDi-diS₀. These two oversulfated disaccharides migrated to the same position on this thin layer chromatography and cannot be separated under these conditions. The further addition of chondro-4-sulfatase to the digestion mixture depleted the detectable ΔDi-4S; however, it did not affect the migration of the oversulfated disaccharide. Treatment with chondro-6-sulfatase markedly depleted the oversulfated [35S]-labeled disaccharide. [35S]ΔDi-diS₀, which was used as control, was susceptible to either one of the sulfatases. Since by using such thin layer chromatography the oversulfated disaccharides could not be separated by migration from ΔDi-diS₀, the chondroitinase ABC-generated disaccharides were analyzed by aminoacyano HPLC. [35S]-Disaccharides eluted in a major peak at a retention time of 8.1 min, corresponding to ΔDi-4S, and a second peak at 12 min, corresponding to ΔDi-diS₀ (Fig. 2a). Treatment of the chondroitinase ABC-digested proteoglycans with chondro-4-sulfatase did not affect the retention time of the disaccharide corresponding to ΔDi-diS₀ (Fig. 2c). However, when these chondroitinase ABC-digested proteoglycans were treated with chondro-6-sulfatase, the peak corresponding to ΔDi-diS₀ disappeared, and a new peak eluted at a retention time of 3.8 min (Fig. 2b). Double digestion of the chondroitinase ABC products with chondro-6-sulfatase and chondro-4-sulfatase resulted in the disappearance of the peak corresponding to ΔDi-4S and ΔDi-diS₀ and the appearance of the new peak at retention time of 3.8 min (Fig. 2d). [35S]ΔDi-diS₀, which was used as control, eluted at a retention time of 15.9 min, and this peak disappeared after treatment with either sulfatase or with both. These results are in accordance with the results obtained from the TLC and therefore indicate that the oversulfated disaccharide derived from LNMC proteoglycan is identical to the chondroitin sulfate D proteoglycan purified from shark cartilage (23). The relative amount of oversulfated disaccharides was determined by comparing total peak cpm values (Fig. 2a) while considering the [35S]/molecule ratio for ΔDi-4S and ΔDi-diS₀, 1:1 and 2:1, respectively. It was calculated that ΔDi-diS₀ contributed 20% ± 2% (n = 3) of the total sulfated disaccharides of the chondroitin sulfate proteoglycan of these cultured cells.

**Proteoglycan Characterization of the Three Fibroblast Monolayers**—Purified [35S]-labeled proteoglycans extracted from the various fibroblast monolayers were analyzed prior to co-culturing with LNMC. As in the LNMC, the proteoglycans were subjected to enzymatic degradation with chondroitinase ABC and AC and to degradation by nitrous acid elimination. Proteoglycan degradation was determined and quantitated by liberation of disaccharides from the macromolecules detected following each procedure by Sephadex G-25/PD-10 chromatography (see Table I). The analysis of the proteoglycans degradation revealed that [35S]-proteoglycans extracted...
Retention times of authentic standards ADi-4S (48.1), ADi-6S (68.3), ADi-dSi (B), ADi-dSi (D), and ADi-dSi (E) run before and after each of the samples are indicated by arrows. Free \( ^{35}S \) is excluded from the column after 24-26 min (not indicated on the graph). The results shown are from one representative experiment out of three.

**TABLE I**

Incorporation of \(^{35}S\)Sulfate into proteoglycan synthesized by mast cells grown on various types of fibroblast monolayers

<table>
<thead>
<tr>
<th>Types of fibroblasts</th>
<th>(^{35}S)Sulfate incorporation (mean ± S.E.; n = 3)</th>
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<tbody>
<tr>
<td>MESF</td>
<td>10,185 ± 1,400 cpmm/10^6 mast cells/4 h</td>
</tr>
<tr>
<td>RESF</td>
<td>175,200 ± 30,000 cpmm/10^6 mast cells/4 h</td>
</tr>
<tr>
<td>3T3</td>
<td>36,150 ± 3,800 cpmm/10^6 mast cells/4 h</td>
</tr>
<tr>
<td></td>
<td>31,460 ± 5,200 cpmm/10^6 mast cells/4 h</td>
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Phenotypic Changes in LNMC Cultured on the Various Fibroblast Monolayers—A comparative analysis was performed among MESF, RESF, and 3T3 fibroblast monolayers on the induction of heparin proteoglycan synthesis in the LNMC. Confluent fibroblast monolayers of all three types were treated prior to the initiation of co-culture with LNMC with a cytostatic dose of x-irradiation (5000 rads). Fibroblast monolayers irradiated as controls showed no proliferation activity, and yet the cells were viable as determined by trypan blue dye exclusion. The amount of \(^{35}S\)Sulfate incorporation into the LNMC macromolecules was determined. LNMC cultured for 30 days on MESF monolayer incorporated 17 times greater \(^{35}S\)Sulfate into macromolecules than the amount observed for LNMC that were not plated on monolayer (Table II), as assessed by PD-10 chromatography. The other two fibroblast monolayers, RESF and 3T3, were found to be less effective in the induction of \(^{35}S\)Sulfate incorporation.

LNMC co-cultured on the various monolayers for 15 and 30 days were separated from the fibroblasts to a purity of more than 90%. \(^{35}S\)-Proteoglycans were then extracted from the mast cells and subjected to chondroitinase AC and nitrous acid treatments. Proteoglycans extracted from LNMC that were separated from co-culture with MESF after 30 days were 93% ± 4% (n = 3) susceptible to nitrous acid elimination (Fig. 3) whereas only 30% ± 7% (n = 3) of the \(^{35}S\)-proteoglycans extracted from LNMC separated from co-culture with 3T3 fibroblasts was degraded by nitrous acid. No degradation by nitrous acid was observed in \(^{35}S\)-proteoglycans extracted from cells separated from co-culture with RESF monolayer.

Although co-cultured LNMC proteoglycans were extracted for analysis from highly purified mast cells, the composition of the MESF fibroblast monolayer's proteoglycan was reanalyzed after the fibroblasts were separated from co-culture with the LNMC. It was found that the post-co-culture MESF proteoglycan composition was identical to that found prior to the co-culture (Table I). More than 80% of the post-co-culture MESF proteoglycan was identified as dermatan sulfate. The amount of incorporation into macromolecules was of the same order as that found prior to the co-culture. During the co-culture period, the fibroblasts maintained their normal histologic appearance with no apparent changes.

Since the MESF was found to be the most potent monolayer in the induction of heparin synthesis in these cells, the kinetics of the shift in proteoglycan types synthesized were determined in LNMC grown on this monolayer (Fig. 4). As seen, the synthesis of chondroitin sulfate D gradually declined whereas that of heparin began 4-7 days after plating and gradually increased. The ratio of 4:1 between ADi-4S and ADi-6S was not changed.
become important phenotypic markers that distinguish different mast cell subsets. Although chondroitin sulfates (ChS-E in mouse bone marrow derived mast cells (2) and ChS-D in rat bone marrow derived mast cells (11)) characterize mast cells of a mucosal phenotype, heparin characterizes mast cells of a connective tissue phenotype (1, 2).

In the present study we have reanalyzed the proteoglycans of LNMC which have already been partially characterized in the past. These mast cells differ from other murine-derived cultured mast cells in their tissue of origin (mesenteric lymph nodes versus bone marrow) and in their morphology. We have previously described preliminary analysis of the granular proteoglycans derived from murine LNMC cultured and prepared in a way similar to that described in this work (19). In this previous study ascending thin layer chromatography was used for the separation of chondroitinase ABC-digested proteoglycans extracted from the LNMC. This enzyme digestion revealed two products that migrated in the positions of ADi-diSa and of oversulfated disaccharides that could be either ADi-diSa or ADi-diSn (25). In that study the chondroitinase ABC products of the LNMC proteoglycans were not treated further by sulfatases, and it was presumed that the oversulfated disaccharides were ADi-diSa. This assumption was based on the identification of that proteoglycan in cultured mouse bone marrow-derived mast cells, which have been well characterized before (2). In the present work, chondroitinase ABC-treated samples were digested further by sulfatases before they were loaded onto the ascending thin layer chromatography plate for analysis. In this TLC procedure we have identified the oversulfated disaccharide as ADi-diSa and not ADi-diSn since chondro-4-sulfatase could not digest the oversulfated material that chondro-6-sulfatase did. To verify our observation and in order that ADi-diSa and ADi-diSn be separated from ADi-diSa we have analyzed further the composition of the LNMC disaccharides by HPLC. In the HPLC, as in the TLC, chondro-6-sulfatase or both sulfatases together but not chondro-4-sulfatase alone was effective in cleaving the sulfated material located on the hexosamine of the disaccharide. Therefore, we concluded that the proteoglycans derived from these LNMC contain disulfated disaccharides GlcUA-2-SO₄ → GalNAc-6-SO₄ (ChS-D). Mouse lymph node-derived mast cell committed precursors, which could not be found in the bone marrow, could be differentiated into connective tissue mast cells in response to conditioned medium derived from fibroblasts (18). This strengthens the idea that the ChS-D-containing LNMC represent a distinct subclass of mast cells. However, it has to be investigated further whether ChS-D-containing LNMC differ by other criteria from the ChS-E-containing mast cells. Another interesting point is whether mast cells have the biochemical machinery to change ChS-D into ChS-E and vice versa.

The present work and studies described previously by us and by others (13–17) emphasize the important role played by fibroblasts in regulating and determining the phenotypic properties of mast cells. Here we have investigated further the fibroblast effect on the synthesis of granule constituents in LNMC.

The procedures for the preparation of RESF and MESF monolayers were well established in the literature (21, 22, 26, 27). These irradiated embryonic skin monolayers of rat and mouse used for maintaining the mast cell differentiation appear as a network of cytoplasm extensions and fibers. Unlike epithelial or macrophage monolayers, these monolayers can be mechanically peeled off as a whole sheet. Additionally, the whole cell population could be destroyed by detergent such as Triton X, leaving behind an extracellular matrix made of continuous fibrous mesh which detached as

**DISCUSSION**

During the last decade oversulfated proteoglycans have become important phenotypic markers that distinguish different mast cell subsets. Although chondroitin sulfates (ChS-E in mouse bone marrow derived mast cells (2) and ChS-D in rat bone marrow derived mast cells (11)) characterize mast cells of a mucosal phenotype, heparin characterizes mast cells of a connective tissue phenotype (1, 2).

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Chondroitin Sulfate D Proteoglycan in Mast Cells

The conversion of ChS-D-containing LNMC into heparin-containing mast cells was time dependent (Fig. 4). Chondroitin sulfate D gradually declined whereas heparin synthesis appeared between 4 and 7 days after plating and gradually increased. Fifty percent of the total 35S-proteoglycans extracted after 15 days was found to be heparin. Whether the same cell synthesizes both types of proteoglycans has to be explored. However, we assume that the shift takes place within the cells and not in populations of cells since it has already been demonstrated by histologic staining in mouse bone marrow-derived mast cells co-cultured with 3T3 fibroblasts that the same cell contains both heparin and chondroitin sulfate proteoglycans (15). The shift of connective tissue mast cells into mucosal type was investigated under in vivo conditions. Somoda and his collaborators (30) have shown the development of mucosal mast cells after injection of a single connective tissue type mast cell in the stomach of genetically mast cell-deficient w/w' mice. This may indicate that the shift from ChS-D into heparin-containing mast cells could be reversible and depend on the appropriate microenvironment.

A strong correlation was observed among the fibroblast density, duration of exposure to monolayer, and histamine content in LNMC. Mast cells that were exposed to optimal concentrations of MESSF contained up to 3 µg of histamine per 10^6 cells and were able to degranulate more than 80% of their histamine content (13).

Mast cell-fibroblast interactions in the co-cultures also include influences exerted on the fibroblasts. Although we did not find evidence for such effects on mast cells on the fibroblasts in this study, other works did. Morphologic studies revealed that the fibroblasts became vacuolated and less adherent to plastic culture dishes when co cultured with mast cells (32). Moreover, mast cell degranulation induced fibroblast contraction and displacement. Fibroblasts lost their contact inhibition and increased their growth rate when cocultured with proliferating interleukin 3-stimulated mast cells (33). This finding may be explained by the latest observation that mast cells release several cytokines in response to immunologic stimuli (34–36), one of which may well be a fibroblast growth factor.

In conclusion, we find that LNMC contain chondroitin sulfate D as their major oversulfated granular proteoglycan. These mast cells may represent a new unfamiliar mast cell subclasse. Like the mouse bone marrow derived mast cells, when co-cultured with fibroblast monolayers, the LNMC shift the synthesis of their granular oversulfated proteoglycan into 100% heparin and change their histology to a more mature mast cell histology. The results indicate that the degree of granule constituent synthesis induced by MESSF is a function of the duration of exposure to and the concentration of the factor(s) produced by the fibroblasts (Fig. 5). These fibroblasts may be considered as a source of cells for further investigating the signal(s) that induce mast cell phenotype changes.

REFERENCES

Chondroitin Sulfate D Proteoglycan in Mast Cells


